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EDITORIAL

Mouse models and the RANKL/OPG axis in myeloma bone disease

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Multiple myeloma is a neoplasm of terminally differentiated plasma cells. The malignant cells proliferate in areas of active hematopoiesis leading to bone destruction, anemia, hypercalcemia, renal dysfunction and hypogammaglobulinemia. Radiological evidence of bone damage is found in 70% of patients with myeloma and is a cause of significant morbidity. Skeletal lesions cause pain, present a risk of pathological fracture and can lead to spinal cord compression. In this issue of *Leukemia*, Rabin *et al.*,[1] describe an animal model of myeloma bone disease that enhances our understanding of the mechanisms behind bone destruction and provides further evidence of the potential therapeutic use of osteoprotegerin (OPG) in preventing this complication. Studies using magnetic resonance imaging have identified three patterns of bone marrow involvement: focal accumulation of cells (multiple plasmacytomas), diffuse marrow infiltration or a mixed pattern of focal and diffuse disease.[2–4] The observation that lytic bone lesions accumulate at sites of active hematopoiesis, highlighted the importance of the bone marrow microenvironment for growth and survival of myeloma cells. The cross talk between myeloma cells and the bone marrow stroma is being dissected out and the interdependence between cells is becoming clearer.[5,6] It is difficult to reproduce fully these cellular interactions *in vitro* and therefore animal models of myeloma are essential. Moreover, these models serve as a platform for testing novel therapeutics for this disease. To put the paper by Rabin *et al.* in context, we will focus on orthotopic models of myeloma that recapitulate bone destruction. Many of these models are based on cell lines injected systemically in immunocompromised animals.[7,8]

The use of human myeloma cell lines provides a convenient supply of cells for animal experiments although proper characterization of the cell lines is imperative.[9–11] In this respect, the current model is based on the KMS-12-BM cell line,[12] which is well characterized and has the t(11;14) cytogenetic abnormality that is often present in myeloma.[13] A common end point in these animal models is infiltration of the vertebral column by tumor cells with the onset of hind-limb paralysis. The first model utilized ARH-77 cells injected systemically in severe combined immune deficiency (SCID) mice. The cells reliably lead to a disease resembling myeloma with detection of a human monoclonal protein, osteolytic lesions and hypercalcemia.[7] However, later studies showed that ARH-77 is a lymphoblastoid cell line due to transformation with the Epstein – Barr virus.[10] Subsequent models based on KPMM2[8] and JJN3[14] cells were devoid of this problem. Apart from lytic bone disease, patients with myeloma can have diffuse osteoporosis. Only two animal models have this feature: one is based on JJN3 cells[14] that secrete the κ light chain and the other is based on the KAS-6/1 cell line[15] that has been genetically modified to express MIP-1α (Dingli *et al.*, manuscript in preparation). When KAS-6/1 cells are injected into irradiated SCID mice, they infiltrate the marrow diffusely without the development of lytic bone disease or osteoporosis. However, when the cells are engineered to express MIP-1α, they induce diffuse osteoporosis, but not focal lytic lesions. This illustrates the importance of the interaction between myeloma cells and the stroma since the cell line was isolated from a patient with lytic bone disease.

In almost all the models, the cells infiltrate other organs such as the spleen, liver and kidneys apart from the bone marrow, a phenomenon that is only seen in patients with advanced myeloma.[7,8,14] However, this is not surprising, given that cell lines are usually obtained from patients with plasma cell leukemia or advanced myeloma, with the myeloma cells isolated from the pleural or peritoneal cavities.[15–17] Presumably, the tumor cells have become independent of the marrow microenvironment for their growth and survival. The KMS-12-BM model exhibits minimal extramedullary spread (liver) and reliably produces lytic bone lesions.[1] The main problem in relation to all these models is that human myeloma cell lines are growing in a murine environment. It is well known that many human plasma membrane receptors do not recognize murine growth factors and vice versa. For example, KAS-6/1 cells are dependent on human IL-6 for growth *in vitro*. Although murine IL-6 does not stimulate the human receptor,[18] KAS-6/1 cells grow well in SCID mice. When the cells are harvested from mice, they remain dependent on human IL-6 supplementation in culture suggesting that the mice are providing them with other growth and survival signals that may or may not be relevant for the tumor in humans. These observations impose limits on the use of these models in understanding the interactions between myeloma cells and the supporting stroma.

In an attempt to provide a human bone marrow environment, SCID mice have been implanted with human fetal bones (SCID-hu) that acquire a vascular supply over a period of weeks. Subsequently, purified primary myeloma cells are injected directly into the bone where they expand and form lytic lesions.[19,20] Although the myeloma cells remain confined within the human bone, the cells circulate in the mouse and will engraft into a second human bone present in the mouse that is not directly injected with the cells. While the SCID-hu may be more realistic, the disease kinetics are slow and very variable, the supply of human fetal bones is limited and the marrow stroma is still allogeneic to the myeloma cells. More recently, NOD/SCID mice harboring human adult bone fragments (NOD/SCID-HAB) that permit growth of the RPMI-8226 human myeloma cell line[21] were reported. Another development was the implantation of human bone marrow biopsies in the hind limb muscles of immunocompromised mice although this creates the need for continued passage of the established tumor in mice.[22] However, these models do not eliminate the problem of human cells in a murine stroma.

All the models discussed require profound immunosuppression for the survival of the injected tumor cells. Therefore, they do not allow studies of the potential immune response against myeloma or the impact of T cells on myeloma growth. The latter may be important given the secretion of receptor activator of NF-κB ligand (RANKL)[23] as well as TNF-related apoptosis-inducing ligand (TRAIL) by activated T cells.[24] Aged C57BL/KaLwRij mice spontaneously developed a disease similar to human multiple myeloma from which various cells (for

example, 5T2 and 5T33MM) were isolated. These cells have to be maintained by serial transplantation in syngeneic mice[25] where they expand, produce a detectable paraprotein and induce lytic bone lesions. The mice ultimately develop hind limb paralysis because of spinal cord compression. This model suffers from the inconvenience imposed by the need to harvest continuously and transplant diseased bone marrow in syngeneic mice. Fortunately, the murine 5TGM1 cell line was recently isolated that can be grown in tissue culture making it much easier to maintain.[26] These cells can be injected in C57BL/KaLwRij mice to develop disease without the need for immunosuppression. In many respects, this is an excellent model since the myeloma cells are in their native environment and the model resembles the human disease very closely, although the tumor cells infiltrate and grow in the spleen.

What have these models taught us about the role of RANKL and OPG in myeloma bone disease and its therapy? Normal bone turnover is maintained by coupling osteoclast-mediated bone resorption with new bone formation by osteoblasts. The presence of myeloma cells within the bone marrow alters the cytokine milieu in favor of osteoclastogenesis. The main culprits seem to be macrophage inflammatory protein 1-α (MIP-1α)[27] and RANKL[23] although interleukin-1β (IL-1β), IL-6, tumor necrosis factor (TNF) α and β may also play direct or indirect roles.[28] RANKL is expressed on the surface of T cells, marrow stromal cells and osteoblasts where it can be cleaved by matrix metalloproteases (and detected as soluble RANK, sRANKL). It stimulates the differentiation and activation of osteoclasts from monocyte – macrophage precursors.[29] Bone marrow stromal cells and osteoblasts produce OPG that acts as a decoy receptor for RANKL damping osteoclast activation. In myeloma, the RANKL/OPG axis is disrupted:[23,30] malignant plasma cells express RANKL and secrete IL-1β, IL-6 and IL-11 that stimulate stromal cells to express more RANKL. Myeloma cells also downregulate OPG production by osteoblasts and endothelial cells[30,31] and high-level expression of CD138 by myeloma cells binds OPG and effectively lowers its concentration, further limiting its ability to neutralize RANKL. In this context, the model presented by Rabin *et al.*[1] provides a semi-quantitative estimate of the correlation between local tumor burden with bone resorption and provides further proof of the importance of the disrupted RANKL/OPG pathway for local bone destruction. These observations from animal models are supported by data from clinical studies. Serum OPG levels are reduced in patients with myeloma and the levels correlate with the number of bone lesions.[31] Similarly, patients with myeloma have high circulating levels of sRANKL.[32] The sRANKL/OPG ratio is an independent prognostic factor for survival in myeloma and can be combined with β2-microglobulin and C-reactive protein into a prognostic scoring system.[32]

Given the importance of the bone marrow microenvironment for myeloma cell growth and survival, there is increasing interest in targeting the RANKL/OPG axis not only to abrogate bone destruction, but also to alter the natural history of the disease. In this context, the available animal models have provided useful platforms to test agents that target this important signaling system. Injection of a fusion protein between murine RANK and the Fc region of human IgG_1 (RANK-Fc) reduced the tumor burden and prolonged the time to hind-limb paralysis in a SCID/ARH-77 model of myeloma.[23] The same fusion protein also reduced the tumor burden in a SCID-hu model with improvement of OPG levels.[23] Native OPG has a half-life of 20 min, but this can be extended by fusion with an immunoglobulin Fc fragment. In the 5T2MM model, Croucher *et al.*[33] showed that OPG-Fc can reduce the number of lytic bone lesions, decrease cancellous bone loss, lower the disease burden based on the serum paraprotein level and increase bone mineral density. Subsequently, using the same animal model, it was shown that OPG-Fc decreases the tumor burden and improved survival by prolonging the time to the onset of hind limb paralysis or cachexia.[34] Unfortunately, patients treated with a human version of OPG-Fc (AMGN-0007) develop neutralizing antibodies, suggesting that repeated administration of the agent may be problematic.[35]

To circumvent the short half life of OPG and prevent the development of neutralizing antibodies, Doran *et al.*[36] used a self-inactivating (SIN) lentiviral vector with OPG under the control of the constitutively active cytomegalovirus promoter to transduce ARH-77 cells. The genetically modified cells can produce OPG continuously for extended periods of time. They provided proof of principle that a continuous supply of OPG could significantly lower the incidence of lytic bone lesions compared to controls. The paper by Rabin *et al.*[1] takes this a step further with the use of human bone marrow mesenchymal stem cells (hMSC) transduced with a SIN vector coding for OPG (MSC^{OPG}). First, the authors describe a model of myeloma bone disease based on the KMS-12-BM cell line. As in other studies, their model shows the importance of close cell-to-cell contact between myeloma and the surrounding stromal cells for local bone destruction. The authors report that when the genetically modified hMSC are injected systemically, the cells preferentially home to sites of myelomatous marrow. When $hMSC^{OPG}$ are injected serially at 2, 3 and 4 weeks after the KMS-12-BM cells, there was a decrease in the number of osteoclasts within the lumbar vertebrae infiltrated by myeloma. The treated mice also had a higher trabecular bone volume compared to controls, suggesting that OPG can reverse the osteoclast/osteoblast imbalance induced by myeloma.

OPG can indirectly serve as a survival factor for myeloma cells by scavenging TRAIL[37] that induces myeloma cell apoptosis.[38,39] Is it possible to dissect out the interaction between OPG, RANKL and TRAIL to optimize therapy? A recent study by Heath *et al.*,[40] using a synthetic OPG-like peptide suggests that OPG may have different binding sites for RANKL and TRAIL and therefore it is in principle possible to inhibit RANKL-mediated bone destruction without interfering with TRAIL-induced myeloma cell apoptosis. In this context, an important outcome of the study by Rabin *et al.* would be the impact on survival in the mice injected with the $hMSC^{OPG}$ cells, but unfortunately this is not reported. In this respect, it is also a pity that the myeloma cell line chosen (KMS-12-BM) does not secrete a paraprotein that would simplify non-invasive monitoring of tumor growth and response to therapy.

We note that the MSC were isolated from healthy human bone marrow donors and injected in mice harboring a human myeloma cell line. One has to consider what can be extrapolated about the homing ability of these hMSC in the context of the human disease. It would be of great interest to see whether the current results hold true in the 5T2MM model using syngeneic MSC engineered to express OPG. Such a model would also allow an evaluation of the durability of OPG expression in an immunocompetent environment. Nonetheless, the current study provides an important advance since, in principle, autologous MSC could be transduced *in vitro*, expanded and injected in patients. Exciting times lie ahead for OPG and its therapeutic applications.

D Dingli[1,2] and SJ Russell[1,2]
[1]*Molecular Medicine Program, Mayo Clinic College of*

Medicine, Rochester, MN, USA and
[2]Division of Hematology, Mayo Clinic College of Medicine, Rochester, MN, USA

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